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The lichen genus *Nephroma* in Australia

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Abstract

A morphological and ehemical investigation of Australian species of *Nephroma* reveals four taxa for the continent: *N. anstrale, N. cellnlosnm, N. helveticnm* and *N. rnfnm*, and clarifies their ehemistry. *Nephroma cellnlosnm* var. *isidioferum* has been reduced to synonymy with *N. cellnlosnm*. The distribution for *N. helveticnm* in Australia is updated and specimens previously identified as such for Victoria and the ACT are in faet *N. rnfnm*. A key to, and some taxonomic notes on, the species are provided.

Introduction

Nephroma Ach. in Luyken, 1809 [type *N. arcticum* (L.) Torss.] derives its name from the Greek *Nephros* (kidney), referring to the shape of the apotheeia, whieh are situated on the lower surface of the lobe apices. Worldwide the genus eomprises about 40 species and these are distributed in the temperate zones of both Northern and Southern Hemispheres (Galloway 1985). Some speeies are tripartite but the majority is bipartite (Lohtander *et al.* 2002). The term bipartite refers to those species that possess only one photosynthetie component, the cyanobacteria *Nostoc*, which more or less forms a continuous photobiont layer under the upper eortex (Lohtander *et al.* 2002). However, in tripartite species two photosynthetic components are present. The green alga *Coccomyxa* forms the photobiont layer under the upper eortex and the cyanobacteria are confined to eephalodia (variously shaped struetures, brain-like in section). These may be present internally in the medulla or externally on the lower surface (Lohtander *et al.* 2002). With the exception of *N. anstrale*, which has *Coccomyxa* as the dominant green photobiont and *Nostoc*-eontaining, external eephalodia, the Australian species are bipartite and contain *Nostoc*.

Nephroma (formerly included in the Peltigeraceae) currently is placed in its own family Nephromataceae (Eriksson & Strand 1995, Lohtander *et al.* 2002, Wetmore 1960) and, indeed, moleeular data suggest that the Nephromataceae are possibly not closely related to the Peltigeraceae (Eriksson & Strand 1995). Seven broad chemical groupings oeeur within the genus (James & White 1987, White & James 1988) and three of these have been identified in Australian species (Groups 4, 5 & 6). One of these chemical groups (Group 4) comprises a series of six triterpenoids that also occur in *Peltigera* and this has previously been interpreted as signifying a close chemical relationship with that genus (James & White 1987). Phylogenetic studies (based on mtSSU rDNA and ITS as well as chemieal characters) demonstrate that *Peltigera* constitutes the sister group to *Nephroma* (Lohtander *et al.* 2002). However, earlier molecular data did not support a elose relationship between the two genera (Eriksson & Strand 1995). As well as chemieal groupings, White & James (1988) provide a detailed discussion on morphotypes, retieulation and propagules in *Nephroma*.

Materials and Methods

Terpenes, depsides and depsidones were identified using thin layer chromatography (TLC) in solvent systems C (toluene/acetic acid – 170:30), G (toluene/ethyl acetate/formic acid – 139:83:8) and EHF (diethyl ether/hexane*/formic acid – 300:100:3)

following techniques outlined in Elix & Ernst-Russell (1993) and Orange *et al.* (2001). * Petroleum spirit b.p.60-80 was used in place of hexane for health and cost considerations. Abbreviations for the terpenes follow those used by White & James (1988). All terpenes appear on the TLC plate as dark purple or brown spots after charring, and as bright orange spots with or without a lighter halo when examined under long UV after charring.

T1 = 7β-acetoxyhopan-22-ol
T2 = 15α-acetoxyhopan-22-ol
T3 = hopane-6α, 22-diol (zeorin)
T4 = hopane-7 β, 22-diol
T5 = hopane-15α, 22-diol

Pigments previously referred to as P1-5 by White & James (1988) have now been identified using high performance liquid chromatography (HPLC; Elix *et al.* 2003) and are shown in Table 1. The colour of the spot, and under long wave UV following charring, is also described there.

Table 1. The identity of pigments 1-5 (as referred to in White & James 1988) and selective TLC data.

Pigments 1-5 (White & James 1988)	Pigments identified by HPLC	Spot colour after acid and charring	UV after charring
P1	*O*-Methylleprolomin	Bright yellow	Bright lime
P2	Exuviatic acid A	Green	Dull brown/orange
P3	Iso-*O*-methylleprolomin	Pale yellow	Bright lime
P4	Exuviatic acid C	Green	Dull brown/orange
P5	Exuviatic acid B	Green	Dull brown/orange

The position of secondary compounds found in Australian *Nephroma* species using solvent system G are shown in Figure 1 and the shading/hatching used is consistent with that of White & James (1988) for comparative purposes.

The morphology and anatomy of almost 250 specimens from BM, BRI, CANB, CHR, FH, H, HO, MEL, NSW and US were examined using light microscopy. This included not only Australian collections but also comparative material from other regions, such as the type collections of *N. cellulosum* from Slaten Island (syntype – BM, isotype – US), *N. cellulosum* var. *isidioferum* J.S.Murray from New Zealand (holotype – CHR), *N. helveticum* from Switzerland (image of lectotype – H) and *Nephromium sublaevigatum* Nyl. (synonym of *Nephroma helveticum*) from Mexico (lectotype – H). Regrettably the type material of *Nephroma australe* from New Zealand (holotype believed to be in PC) and that of *N. rufum* from New Zealand (lectotype – BM, isolectotype – VER) could not be traced. Ascospores were examined and measured in hand-cut sections mounted in water or dilute KOH for clarity. Pycnoconidia (asexual spores) were examined and measured using a diluted erythrosine solution, which greatly reduces their movement and stains them red, thereby improving clarity.

Key to the Species

1 Thallus tripartite; photobiont green but internal cephalodia containing blue-green algae (*Nostoc*) also present; thallus yellow to green or occasionally olive..... ..... *N. australe*

Thallus bipartite; photobiont blue-green; cephalodia absent; thallus red-brown or greyish-brown or bluish 2

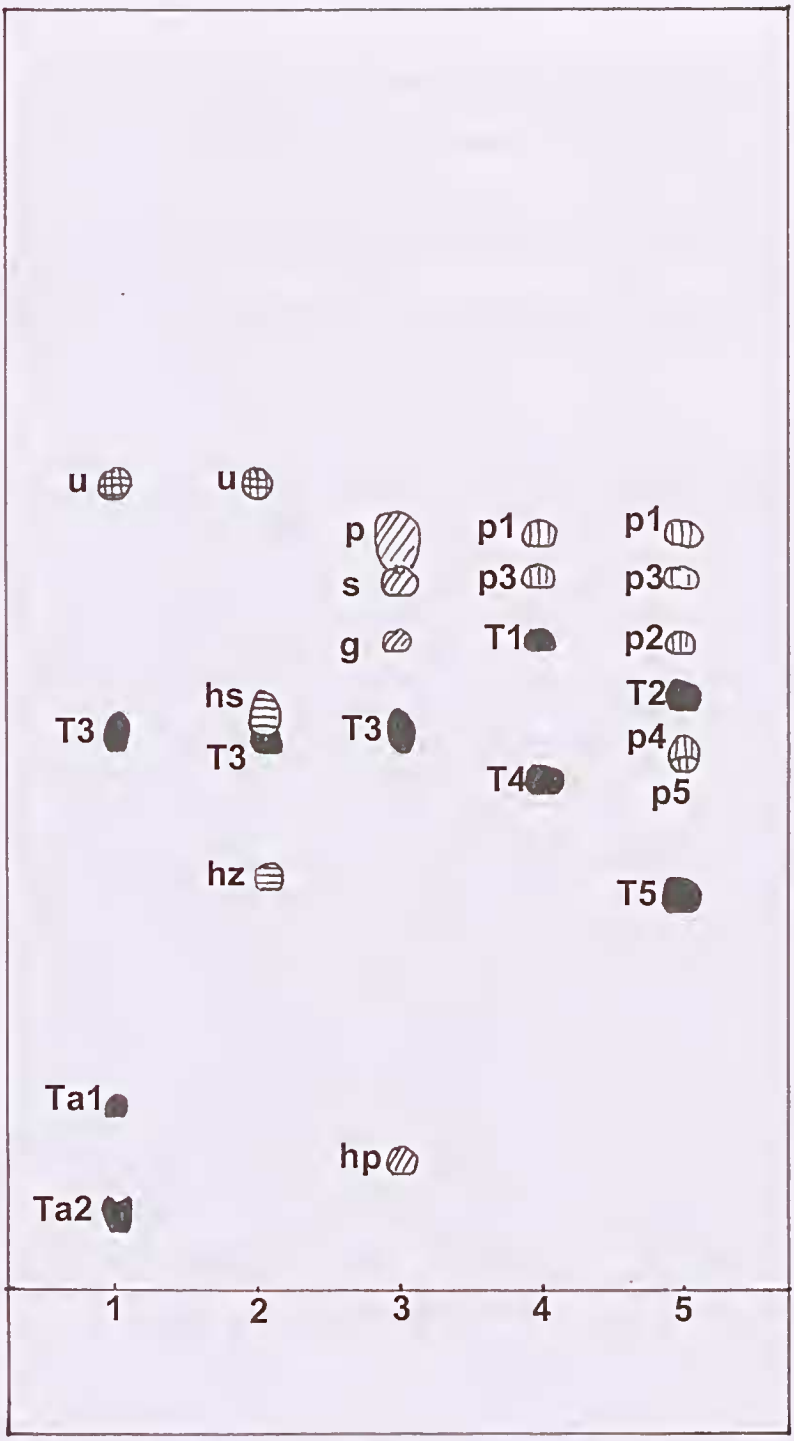

Figure 1. Representation of TLC plate showing position of secondary compounds found in Australian *Nephroma*, using solvent system G. Spots 1-5: 1 = *N. australe* race ii; 2 = *N. australe* race i; 3 = *N. cellulosum*; 4 = *N. helveticum*; 5 = *N. rufum*. P1-P5 are pigments (see Table 1), T1-T5 are terpenes (see materials and methods), Ta1 and Ta2 are associated terpenes (see text *N. australe*). u = usnic acid, hz = hyposalazinic acid, hs = hypostictic acid, p = perlatolic acid, s = stenosporic acid, g = glomelliferic acid, hp = hydrolysis product of perlatolic acid.

2 Upper surface strongly faveolate-reticulate; lower surface white, bullate ....*N. cellulosum*
Upper surface even or weakly depressed; lower surface dark brown or black, undulate 3

3 Thallus margins denticulate, ± flattened, with elongate, mostly terete phyllidia, rarely extending to upper surface; lower surface tomentose; dorsal surface of apothecia scabrid.....*N. helveticum*
Thallus margins not denticulate; upper surface and margins with scattered or clustered, ± flattened phyllidia; lower surface glabrous or occasionally ± subpubescent; dorsal surface of apothecia smooth and faveolate.....*N. rufum*

The Species

1. *Nephroma australe* A. Rich., *Voy. Astrolabe Bot. Pars* 1: 31 (1832)

A detailed description of this species is given in White & James (1988) and Galloway (1985) and it will be treated in the next *Flora of Australia* lichen series.

Nephroma australe is readily distinguished from the other Australian species by the yellowish or greenish brown (indicative of usnic acid), smooth upper surface and by the tripartite nature of the thallus with the dominant green photobiont *Coccomyxa* and *Nostoc*-containing cephalodia. The growth habit varies considerably depending upon the substratum but typically the sterile lobes are flat and ±attached and the fertile lobes are robust, imbricate, upturned and growing away from the substratum.

White & James (1988) recognise two chemical races, referred to by Galloway (1985) as chemodemes (for New Zealand populations), based on the presence or absence of depsidones (Moroney *et al.* 1981). The majority of Australian specimens belong to chemical race ii (Figure 1), containing (±) usnic acid, hopane-6α, 22-diol (major), 2 unidentified terpenoids with low Rf (minor), (±) additional unidentified terpenes (trace). Despite being present in significant amounts and consistently detectable by TLC, the structure of the two terpenes with low Rf has not been determined (J.A. Elix, pers. comm.). Only two specimens, both from Tasmania (HO 525243, HO 517563), belong to chemical race i (Figure 1) and contain usnic acid, hopane-6α, 22-diol (major), hypostictic acid (minor) and hyposalazinic acid (minor). Interestingly, these two collections are from dolerite rocks whereas the other Tasmanian collections, with chemical race ii, were corticolous or from other rock types. Additional chemical variants (e.g. with additional stictic acid) have been reported (from New Zealand and Tasmania) but these are uncommon and have been omitted here, as they do not always give reproducible results (see also Galloway 1985, White & James 1988). The size of the ascospores reported by White & James (1988) are somewhat larger than those measured for the Australian specimens in the present study [(22-)24-28 x 7.5-8.5 µm cf. 17-20(-22) x 5-7.5 µm].

Representative specimens examined (total 42): **NEW SOUTH WALES**: Northern Tablelands, headwaters of Edwards Creek, Barrington Tops, 32°04'S, 151°33'E, on *Leptospermum* sp., 28.xii.1965, *R.B. Filson 8023* (MEL 1021147); along road from Barrington to Seone, 1350 m altitude, on *Trochocarpa* sp. in rainforest, 4.vii.1988, *G. Kantvilas 466/88* (NSW 231155); **TASMANIA**: Montezuma Falls Track, Williamsford end, 41°51'S, 145°30'E, 320 m altitude, on *Nothofagus* in shelter, 4.i.1974, *G.C. Bratt 74/68* (HO 37304); lower slopes of Drys Bluff, 41°41'S, 146°49'E, 500 m altitude, on dolerite rocks in open eucalypt forest, 23.vi.2002, *G. Kantvilas 333/02* (HO 517563); North West, Circular Head, 40°46'S, 145°18'E, *Weymouth 34* (MEL 7368); **VICTORIA**: East Gippsland, Goonmirek Rocks, Errinundra Flora Reserve, 37°17'S, 148°53'E, 1200 m altitude, growing on *Podocarpus lawrencii* in cool temperate rainforest, 29.iii.1988, *J.A. Elix 21890* (MEL 1064421); Eastern Highlands, Federation Track between Mt Donna Buang and Mt Boobyalla, growing on *Eucalyptus pauciflora*, 21.iii.1965, *R.B. Filson 7042* (MEL 35077).

Nephroma australe is a widespread, austral species occurring in the cool temperate rainforests of Australasia and southern South America (Galloway 1985, White & James

1988). In Australia it is known from New South Wales, Victoria and Tasmania (McCarthy 2003), and it is most common and shows greatest diversity in the last state. *Nephroma australe* is corticolous, saxicolous and muscicolous, occurring in cool temperate rainforests.

2. *Nephroma cellulosum* (Sm. ex Ach.) Ach., *Lichenogr. Universalis*: 523 (1810)

Nephroma cellulosum var. *isidioferum* J.S. Murray, *Transactions of the Royal Society of New Zealand* 88: 285 (1960). Type: New Zealand, Otago, Flagstaff, *J.S. Thomson*, 1884 (Holotype – CHR!).

A detailed description of this species is given in White & James [(1988) as *N. cellulosum* var. *cellulosum*] and Galloway (1985) and it will be treated in the next *Flora of Australia* lichen series.

This species is characterised by the robust, orbicular to spreading thallus with olive-brown to reddish-brown, greyish-red or grey (depending on exposure to sunlight), markedly faveolate and ridged upper surface, with or without terete to squamiform phyllidia along the ridges between faveolae, and by the bullate lower surface with whitish, shining margins. The medullary substances include hopane-6α, 22-diol (minor), perlatolic acid (major), glomelliferic acid (major), stenosporic acid (minor), (±) additional traces of glomellic, anziaic and loxodellic acids that are detectable by HPLC but are not always visible on a TLC plate. HPLC has confirmed that the additional low Rf spots sometimes apparent on TLC plates using solvent G (Figure 1) are hydrolysis products of perlatolic acid (J.A. Elix, pers. comm.), as suggested in White & James (1988). The latter authors also report the presence of an occasional yellow pigment in the medulla (K+ purple) but these have not been observed in Australian specimens.

Nephroma cellulosum var. *isidioferum* (syn. *N. lepidophyllum* Räs. ex Gyeln.) has been regarded as distinct from *N. cellulosum* var. *cellulosum* (Galloway 1985, White & James 1988) due to the presence of coarse, terete to squamiform phyllidia along ridges between faveolae, as well as along the margins of lobes and apothecia, and also by an overall greyish bloom in central areas of the thallus (White & James 1988). However, upon closer examination these varieties were found to intergrade in the Australian specimens. The ridges separating the faveolae may become very sharp and this often leads to the cortex rupturing, revealing the medulla. Flatter ridges are less pronounced and do not rupture but are usually still distinctly maculate. Small, terete or broad squamiform phyllidia often, but not always, develop from cracks and maculae on the upper surface. These phyllidia or squamules are considered to be regenerative in nature. They often become undulating and fringed and may develop into young lobes. As many species of *Nephroma* may have them the author follows Wetmore (1960) in considering this character to have little taxonomic significance in the genus. Field studies in Valdivia, Chile by P.W. James indicated that there is a continuum in mixed populations on *Nothofagus* species, with var. *isidioferum* differing only in the production of phyllidia and suppression of apothecia (White & James 1988), which was why these authors only afforded the two taxa varietal status. The type material of *N. cellulosum* does not possess the phyllidiate ridges, whereas that of var. *isidioferum* does. However, it should be noted that much of the type material of *N. cellulosum* was mounted upside down and, in addition, that the specimens were fragmented. White & James (1988) suggest that abundantly fertile material previously determined as var. *cellulosum*, with clustered or scattered phyllidia that are often coralloid, could, in part, also be assigned to var. *isidioferum*. These frequent observations of apparent intermediates, both by this author and others (G. Kantvilas, pers. com., White & James 1988), the regenerative nature of the phyllidia, and the fact that there does not appear to be any difference in distribution and ecology (Galloway 1985 (as *N. lepidophyllum*), Kantvilas & Elix 1992, White & James 1988) or chemistry, suggests that the two varieties form part of the natural variation displayed by *N. cellulosum*.

A preliminary examination of collections from New Zealand, determined as *N. lepidophyllum*, shows that the overall appearance corresponds to that of the Australian

material of *N. cellulosum*. White & James (1988) consider *N. lepidophyllum*, as well as *N. lepidophyllum* f. *hypomelaena* Räs. ex Lamb, to be synonyms of *N. cellulosum* var. *isidioferum*, however, the type material of these two synonyms was not seen for the present study.

Representative specimens examined (total 127): **NEW SOUTH WALES**: Buddong Creek, Forest Way, Bago State Forest, 35°41'S, 148°10'E, 1200 m altitude, on exposed *Eucalyptus*, broad swampy ck, 3.x.1999, *H. Streimann 65327* (CANB 610308.1); Northern Tablelands, headwaters of Edwards Creek, Barrington Tops, 32°04'S, 151°33'E, on *Leptospermum* sp., 28.xii.1965, *R.B. Filson 8022* (MEL 1021144); Swampy Plains River, below Mt. Kosciuszko, 14.ii.1968, *W.A. Weber & D. McVean* s.n. (NSW 524341); **TASMANIA**: Wombat Hill, 41°29'S, 145°27'E, 690 m altitude, on *Cassinia aculeata* in wet scrub at edge of rainforest, 7.xi.1991, *G. Kantvilas et al. 387/91* (HO 63657); near Parrawe, 41°19'S, 145°35'E, on *Cassinia* in shelter, 25.iv.1973, *G.C. Bratt 73/395* (HO 37215); South West, Florentine Valley, 42°40'S, 146°27'E, 17.ii.1974, *M. Westbrook 74/434* (HO 520371); **VICTORIA**: Mt Macedon, v.1885, *F.R.M. Wilson* s.n. (NSW 524344); Midlands, Mt Macedon, Stoney Creek, 37°22'S, 144°34'E, corticolous in rainforest, 26.ix.1987, *R.B. Filson 10307* (MEL 1067038).

Nephroma cellulosum is a widespread austral species occurring in southern temperate South America, including Islas Juan Fernández (Galloway 1985, Redón & Quilhot 1977) and New Zealand (White & James 1988). In Australia it is known from New South Wales, Victoria and Tasmania (Kantvilas & Elix 1992, McCarthy 2003) where it occurs in rainforest, heathland, and high altitude mixed selerophyll and open *Eucalyptus* forest. It is common on corticolous, saxicolous or muscicolous substrata.

3. *Nephroma helveticum* Ach., *Lichenogr. Universalis*: 523 (1810)

A detailed description of this species is given in White & James (1988) and Galloway (1985) and it will be treated in the next *Flora of Australia* lichen series.

This species is characterised by the brown, greyish-brown or pale grey, pubescent upper surface, the predominantly marginal phyllidia and the black or dark brown, tomentose lower surface. It contains hopane-7β, 22-diol (major), (±) 7β-acetoxyhopan-22-ol (minor/trace) and (±) minor amounts or traces of pigments (Figure 1) previously referred to as P1 & P3 (White & James 1988). The nature of these was determined using HPLC and identified as *O*-methylleprolomin (minor/trace: P1), iso-*O*-methylleprolomin (minor/trace: P3). In addition (±) traces of methyl gyrophorate have been detected.

Nephroma helveticum forms a cosmopolitan species aggregate (James & White 1987, White & James 1988, Lohtander *et al.* 2002), which is currently undergoing investigation (White & James 1988). One of the taxa that has been segregated is *N. rufum* (Galloway 1983), distinguishable by phyllidia that initially develop laminally rather than marginally (although they may spread to the margins subsequently), by the non-pubescent upper surface, the glabrous (or rarely subpubescent) lower surface and by the alternative chemistry (terpenes T2 & T5 instead of T1 & T4). *Nephroma helveticum* has been reported to have larger spores than *N. rufum* (15-20 µm *cf.* 20-25 µm) but the spore size did not appear to vary greatly among the Australian specimens.

Representative specimens examined (total 7): **NEW SOUTH WALES**: New England National Park, Point Lookout, 30°29'S, 152°25'E, on *Eucalyptus* trunk on edge of *Nothofagus* forest, x.1967, *D. McVean 67199* (CANB 00578038); Northern Tablelands, headwaters of Edwards Creek, Barrington Tops, 32°04'S, 151°33'E, 28.xii.1965, *R.B. Filson 8022* (MEL 2266605); Central Tablelands, Blue Mountains, 33°30'S, 150°22'E, 310 m altitude, in rainforest country, 26.x.1965, *R.B. Filson 7499* (MEL 1021138); **QUEENSLAND**: Darling Downs, Toowoomba, 27°34'S, 151°57'E, *Hartmann s.n.* (MEL 7370)

Nephroma helveticum is a cosmopolitan species, uncommon in Australia and reported from Queensland, New South Wales, Victoria and the Australian Capital Territory (McCarthy 2003). In the present work this species has been confirmed only for Queensland (rare) and New South Wales. Former records from the Australian Capital

Territory and Victoria are identified as *N. rufum*, which appears to be the more common of the two species. *Nephroma helveticum* is known to be both corticolous and saxicolous but in Australia it has been observed only as growing on bark and twigs.

4. *Nephroma rufum* (C.Bab.) P.James, *New Zealand J. Bot.* 21: 195 (1983)

A detailed description of this species is given in White & James (1988) and Galloway (1985) and it will be treated in the next *Flora of Australia* lichen series.

This species is characterised by the dark reddish-brown, greyish-brown or grey, smooth or ± ridged-faveolate, phyllidiate upper surface and dark brown to black, glabrous or subpubescent lower surface. It contains hopane-15α, 22-diol (major), (±)15α-acetoxyhopan-22-ol (minor/trace), and (±) minor amounts or traces of pigments (Figure 1) previously referred to as P1-5 (White & James 1988, p160). Two of these, *O*-methylleprolomin (P1) and iso-*O*-methylleprolomin (P3), also occur in *N. helveticum* but the exuviatic acids A, B & C (P2, P5 & P4 respectively) have been identified only for *N. rufum*. Although exuviatic acid C (P4) was reported as common for *N. helveticum* (White & James 1988), it was observed only for Australian specimens of *N. rufum*. Methyl gyrophorate and gyrophoric acids were detected in some specimens of *N. rufum* although this was not reported by White & James (1988). *N. rufum* resembles *N. helveticum* but the two are separated by the nature of the lower and upper surfaces, the distribution of the phyllidia and by their chemistry.

Representative specimens examined (total 59): **AUSTRALIAN CAPITAL TERRITORY**: Southern Tablelands, Booroomba Rocks, 35°33'S, 148°58'S, 22.ii.1984, *R.B. Filson 19330* (MEL 1048959); Southern Tablelands, along Gibraltar Creek near Smoker's Gap, 35°31'S, 148°55'E, 1140 m altitude, on *Eucalyptus* sp., 25.xi.1975, *J.A. Elix 1382* (MEL 1017188); **NEW SOUTH WALES:** Brown Mountain, 36°36'S, 149°23'E, 850 m altitude, on granite rocks in wet sclerophyll forest, 6.xii.1978, *J.A. Elix 5459* (CANB 9615083); 5 km NE of Nerriga, along banks of Endriek River, 35°06'S, 150°05'E, 550 m altitude, on shaded sandstone rocks in dry sclerophyll forest, 30.iii.1977, *J.A. Elix 3123* (CANB 9615082); **QUEENSLAND**: Darling Downs, Killarney, 26°46'S, 151°25'E, on tree *F.R.M. Wilson* s.n. (MEL 7369); **VICTORIA**: Otway Ranges, Lorne, 38°31'S, 143°58'E, on rock, *F.R.M. Wilson* s.n. (MEL 7367); **TASMANIA**: Stanhope Colliery near Avoca, 41°43'S, 147°39'E, on dolerite in slight shelter, 25.xi.1970, *G.C. Bratt 70/1392* (HO 37311); Elephant Pass, 41°38'S, 148°14'E, on soil and mudstone in exposed situation, 14.iii.1970, *G.C. Bratt 70/351* (HO 65391).

Nephroma rufum is an Australasian species reported for New Zealand and Australia (Galloway 1985) where it occurs in the Australian Capital Territory, Queensland, New South Wales, Victoria and Tasmania (McCarthy 2003). It most commonly grows on rocks, often among mosses, but also on soil, tree trunks and branches.

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